Ping Kuen Lam, et al. Application No.: 09/365,677

Page 11

The combination of the inventive CBSG material including keratinocytes and dermal fibroblasts and INTEGRATM artificial skin material was applied to a 28-year old man with extensive hypertrophic burn scars and neck contracture. Multiple wounds from scar excision with a total area of approximately 250 cm² were covered with INTEGRATM artificial skin material. At the same time a skin biopsy was taken for keratinocyte culture. Two weeks later, the top silicone layer of the artificial skin was removed. The neodermis of the artificial skin was then covered with the CBSG. At Days 6 and 14 after the application of CBSG, biopsies were taken from center of one of the wounds. The histology at Day 6 showed early epithelization with a few squamous epithelial cells settling on top of the scaffolds of the INTEGRATM artificial skin material and the LASERSKINTM artificial skin material. Most of the epithelium were single-layer flattened squamous cells. The biopsy taken on Day 14 showed a viable 2- to 4-cell-thick squamous epithelium with minimal inflammation. The wounds were completely epithelized within four weeks of the application of the CBSG. Biopsies taken from other wounds showed similar findings.

IN THE CLAIMS:

Please cancel claims 7, 14, and 16 without prejudice.

Please amend claims 1, 4, 8, 11, and 15 to read as follows:

1. (Twice Amended) A method for cultivating a graftable skin material for application to a human patient, said method comprising:

growing a layer of human dermal fibroblasts upon an upper side of a biosynthetic substratum of an esterified hyaluronic acid; and, after said dermal fibroblast layer begins to proliferate,

growing a layer of human keratinocytes harvested from said patient over said dermal fibroblasts upon said upper side of said substratum to form said graftable skin material, said material thereby comprising viable human dermal fibroblasts and viable human keratinocytes on said upper side.

4. (Twice Amended) A method for cultivating graftable skin material for application to a human patient, said method comprising:

growing a first ayer of human dermal fibroblasts upon a basal side of a biosynthetic substratum of an esterified hyaluronic acid;

But Out

Ping Kuen Lam, et al. Application No.: 09/365,677

Page 12

SUBDE SUBDE growing a second human dermal fibroblast layer upon an upper side of said biosynthetic substratum; and

after said second dermal fibroblast layer begins to proliferate, growing a layer of keratinocytes over said second layer to form a composite skin material, said keratinocytes having been harvested from said patient.

5m 08)

8. (Twice Amended) A graftable skin material for application to a patient, said material comprising a composite of:

a biosynthetic substratum of an esterified hyaluronic acid;

a layer of viable human dermal fibroblasts upon an upper side of said biosynthetic substratum; and

a layer of viable human keratinocytes over said dermal fibroblasts upon said upper side of said substratum, said keratinocytes having been harvested from said patient.

11. (Twice Amended) A graftable skin material for application to a patient, said material comprising a composite of:

material comprising a

a biosynthetic substratum of an esterified hyaluronic acid;

a first layer of viable human dermal fibroblasts upon a basal side of said biosynthetic substratum;

a second layer of viable human dermal fibroblasts upon an upper side of said biosynthetic substratum; and

a layer of viable human keratinocytes over said dermal fibroblasts upon said upper side of said substratum, said keratinocytes having been harvested from said patient.

15. (Twice Amended) A method for grafting a graftable skin material onto a human patient, comprising the steps of:

applying an artificial skin substrate upon a wound bed of said patient; said artificial skin substrate comprising a layer of collagen-glycoaminoglycan on a basal side to be juxtaposed to said wound bed and a covering membrane of silicone on an opposing upper side;

allowing a vascularized wound bed to form under said collagen-glycoaminoglycan; thereupon

removing said silicone memorane; and

50B D8)

DIT

Ping Kuen Lam, et al. Application No.: 09/365,677 Page 13

But

applying a basal side of a sheet of cultivated skin material over said collagenglycoaminoglycan, said cultivated skin material comprising a layer of keratinocytes overlying a layer of viable human dermal fibroblasts upon an upper side of a biosynthetic substratum, said keratinocytes being harvested from said patient.

Please add the following new claims 18-30:

- --18. (New) A method according to claim 15, wherein said biosynthetic substratum is a substratum of an esterified hyaluronic acid.
- 19. (New) The method according to claim 18 wherein said cultivated skin material further comprises a layer of dermal fibroblasts upon an upper side of said biosynthetic substratum and wherein said layer of keratinocytes is over said dermal fibroblasts.
- 20. (New) The method according to claim 19 wherein said cultivated skin material further comprises a layer of dermal fibroblasts upon said basal side of said biosynthetic substratum.
 - 21. (New) The method of claim 1, wherein the esterified hyaluronic acid is benzyl esterified hyaluronic acid.
 - 22. (New) The method of claim 4, wherein the esterified hyaluronic acid is benzyl esterified hyaluronic acid.
 - 23. (New) The material of claim 8, wherein the substratum is a membrane comprising benzyl esterified hyaluronic acid.
 - 24. (New) The material of claim 11, wherein the substratum is a membrane comprising benzyl esterified hyaluronic acid.
 - 25. (New) The method of claim 18, wherein the substratum is a membrane comprising benzyl esterified hyaluronic acid.
 - 26. (New) The method of claim 21, wherein the membrane has microholes of about 40 μm diameter.